



Research paper

Redox imbalance and mitochondrial abnormalities in the diabetic lung



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ABSTRACT

Although the lung is one of the least studied organs in diabetes, increasing evidence indicates that it is an inevitable target of diabetic complications. Nevertheless, the underlying biochemical mechanisms of lung injury in diabetes remain largely unexplored. Given that redox imbalance, oxidative stress, and mitochondrial dysfunction have been implicated in diabetic tissue injury, we set out to investigate mechanisms of lung injury in diabetes. The objective of this study was to evaluate NADH/NAD⁺ redox status, oxidative stress, and mitochondrial abnormalities in the diabetic lung. Using STZ induced diabetes in rat as a model, we measured redox-imbalance related parameters including aldose reductase activity, level of poly ADP ribose polymerase (PARP-1), NAD⁺ content, NADPH content, reduced form of glutathione (GSH), and glucose 6-phosphate dehydrogenase (G6PD) activity. For assessment of mitochondrial abnormalities in the diabetic lung, we measured the activities of mitochondrial electron transport chain complexes I to IV and complex V as well as dihydrolipoamide dehydrogenase (DLDH) content and activity. We also measured the protein content of NAD⁺ dependent enzymes such as sirtuin3 (sirt3) and NAD(P)H: quinone oxidoreductase 1 (NQO1). Our results demonstrate that NADH/NAD⁺ redox imbalance occurs in the diabetic lung. This redox imbalance upregulates the activities of complexes I to IV, but not complex V; and this upregulation is likely the source of increased mitochondrial ROS production, oxidative stress, and cell death in the diabetic lung. These results, together with the findings that the protein contents of DLDH, sirt3, and NQO1 all are decreased in the diabetic lung, demonstrate that redox imbalance, mitochondrial abnormality, and oxidative stress contribute to lung injury in diabetes.

1. Introduction

Diabetes is a problem of glucose metabolism and diabetes complications is the outcome of glucose toxicity, which is often manifested by increased protein glycation, activation of the polyol pathway and poly ADP ribose polymerase (PARP), and protein kinase C activation [1–5]. Mechanistically, all these hyperglycemia upregulated pathways can eventually lead to production of reactive oxygen species (ROS) that then induce oxidative stress, mitochondrial dysfunction, and cell death [6,7]. Although the lung is one of the least studied organs in diabetes complications, increasing evidence has indicated that the lung is a target of diabetic injury [8–11]. Nevertheless, the underlying mechanisms remain largely unknown.

As glucose is one of the major sources of NADH, its excess can often lead to excess NADH production and NAD⁺ deficiency, thereby causing NADH/NAD⁺ redox imbalance [12]. The major source of this redox imbalance is thought to come from the activation of the polyol pathway and poly ADP ribose polymerase (PARP) [13–16]. On one hand, the

polyol pathway converts NADPH to NADH when it transforms glucose to fructose via a two-reaction mechanism [17], resulting in NADH overproduction at the consumption of glucose [18,19]. On the other hand, as PARP uses NAD⁺ as its substrate and is usually over-activated by DNA oxidative damage in diabetes [20], cellular NAD⁺ could be potentially depleted [21–23]. Therefore, the overall outcome of the two activated pathways is NADH/NAD⁺ redox imbalance with diminished levels of NAD⁺ and increased levels of NADH, leading to reductive stress that gradually progresses to oxidative stress [24].

Oxidative stress occurs when cellular antioxidative system is defeated by ROS that are overproduced under a variety of disease conditions including diabetes [25]. As mitochondrion is a major source of ROS and a target of ROS [26,27], its abnormalities have been thought to contribute to diabetic pathogenesis [28]. However, whether mitochondrial abnormalities also occur in the diabetic lung remains to be evaluated. In the present study, using STZ induced diabetes in rat as a model; we characterized pulmonary redox imbalance and its associated pathways. Specifically, we measured the activities of mitochon-

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drial membrane complexes I to V. We also measured the enzyme activities of mitochondrial dihydrolipoamide dehydrogenase (DLDH) and its possible modifications by protein acetylation. Additionally, lung mitochondrial ROS production and overall protein oxidative damage were quantified. NAD(P)H: quinone oxidoreductase 1 (NQO1) protein content and activity and sirtuin 3 (sirt3) protein content were also evaluated in the context of redox imbalance and mitochondrial abnormalities in the diabetic lung.

2. Materials and methods

2.1. Chemicals

Biotin-linked aldehyde reactive probe ARP) for protein carbonyl assay was from Cayman Chemical (Ann Arbor, MI). Dihydrolipoamide was synthesized from lipoamide in our own laboratory using sodium borohydride as previously described [29,30]. ϵ -amino-*N*-caproic acid was obtained from MP Biochemicals. Acrylamide/bisacrylamide, ammonium persulfate, Bradford protein assay solution, coomassie brilliant blue (CBB) R-250, immunoblotting membrane, and an ECL immunochemical detection kit were from Bio-Rad laboratories (Richmond, CA, USA). NADH, BSA, lipoamide, EDTA, ATP, and NBT chloride tablets were obtained from Sigma (St. Louis, MO, USA). Serva Blue G was purchased from Serva (Heidelberg, Germany). Anti-PARP antibody was purchased from Trevigen (Gaithersburg, MD). Anti-NQO1 antibodies were from Sigma. Rabbit anti-DLDH polyclonal antibodies (IgG) and goat anti-rabbit IgG conjugated with horseradish peroxidase were purchased from US Biological (Swampscott, MA, USA) and Invitrogen (San Diego, CA, USA), respectively. Other antibodies were from Abcam (Cambridge, MA).

2.2. Diabetes induction in rats

Young adult male Sprague Dawley rats obtained from Charles River were used in this study. Diabetes was induced by a single intraperitoneal injection of STZ (60 mg/kg body weight) into rats weighing 220–250 g after overnight fasting as previously described [31]. STZ solutions were made fresh by dissolving in 0.1 M sodium citrate buffer (pH 4.5) and control animals received sodium citrate buffer only. Blood glucose concentration was monitored once a week using blood glucose test strips (FreeStyle lite from Abbott Diabetes Care Inc., Alameda, California). Rats with blood glucose contents exceeding 200 mg/dl were deemed diabetic. Four weeks post STZ injections, rats were sacrificed and tissues were collected. All animal studies procedures were approved by the UNTHSC committee for research.

2.3. Isolation of lung mitochondria

Mitochondria from the lung were isolated according to a previously described method [32] with slight modifications. Essentially, lung tissues were homogenized (1g/10 ml isolation buffer) in mitochondrial isolation buffer containing 15 mM MOPS (pH 7.2), 70 mM sucrose, 230 mM mannitol, and 1 mM K^+ -EDTA. The homogenates were centrifuged at 800g for 10 min at 4 °C. The resulting supernatant was further centrifuged at 8,000g for 10 min also at 4 °C. The resulting pellet containing crude mitochondria was washed with 10 ml of the isolation buffer followed by centrifugation under the same conditions. The obtained mitochondrial pellet was either used immediately or frozen at –80 °C until use.

2.4. Measurement of H_2O_2 and protein carbonyls

Lung tissue homogenate H_2O_2 was measured by the Amplex Red method [33] using a kit purchased from Invitrogen (catalog number A22188). Protein carbonyls of whole mitochondrial preparation were measured by derivatization with biotin-linked aldehyde reactive probe

(ARP) [34] followed by SDS-PAGE resolution of the carbonylated proteins and Western blot assay and densitometric quantification of each gel lane.

2.5. Measurement of $NAD^+/NADH$ ratio, NADPH, and ATP

Lung tissue homogenate $NAD^+/NADH$ ratio was measured spectrophotometrically by following the changes at 340 nm using a kit from BioAssay (Hayward, CA). NADPH content was measured by a kit from BioVision (Milpitas, CA, Catalog number: K347-100) according to the manufacturer's instructions. ATP content was determined colorimetrically by the ATP Colorimetric/Fluorometric Assay kit that is also from BioVision (Milpitas, CA, catalog number K354-100). This method quantifies phosphorylated glycerol that can be easily monitored at 570 nm.

2.6. Measurement of enzyme activities

Aldose reductase activity was measured spectrophotometrically by following the decrease of NADPH's absorption at 340 nm as previously described [35]. DLDH dehydrogenase activity was measured in the forward reaction as previously described [36,37]. Measurement of mitochondrial complexes I, IV and V activities was also conducted as previously described using in-gel based assays or spectrophotometric assays [38]. Activities for complexes II and III were measured spectrophotometrically as previously described [39,40]. NQO1 activity was measured according to the method of Lind et al. [41] and G6PD activity was measured by monitoring NADPH production at 340 nm as previously described [42]. Caspase-3 activity was measured using a kit also from BioAssay (Hayward, CA). Mitochondrial membrane potential was measured by a kit purchased from BioVision (Milpitas, CA) according to the manufacturer's instruction manual.

2.7. Polyacrylamide gel electrophoresis and Western blot analysis

SDS-PAGE (typically 10% resolving gel) was performed according to standard procedures [43]. One of the resulting gels was stained with Coomassie colloid blue [38], and the other gel was subjected to electrophoretic transfer to membrane for immunoblotting [44]. Signals on the immunoblotting membrane were visualized with an enhanced chemiluminescence kit. Nongradient blue native gel electrophoresis (BN-PAGE) was performed as previously described [36]. All images were scanned by an EPSON PERFECTION 1670 scanner. All densitometric quantifications of gel images were analyzed by AlphaEaseFC software.

2.8. Data analysis

Where appropriate, all values were presented as mean \pm SEM. Statistical data analysis was performed using GraphPad's 2-tailed unpaired *t*-test (GraphPad, San Diego, CA). A *p* value less than 0.05 (*p* < 0.05) was deemed statistically significant.

3. Results

3.1. Redox imbalance in the diabetic lung

In many diabetic tissues that have been well studied, redox imbalance between NADH and NAD^+ is the primary driving force for ROS production and oxidative stress [12,13]. This redox imbalance is believed to mainly originate from two enzyme systems activated by persistent hyperglycemia. One reaction is the polyol pathway including aldose reductase and sorbitol dehydrogenase [45]. This pathway converts glucose to fructose and NADPH to NADH, resulting in overproduction of NADH [46]. Another pathway is poly ADP ribose polymerase (PARP) that uses NAD^+ as its substrate [47]. This enzyme

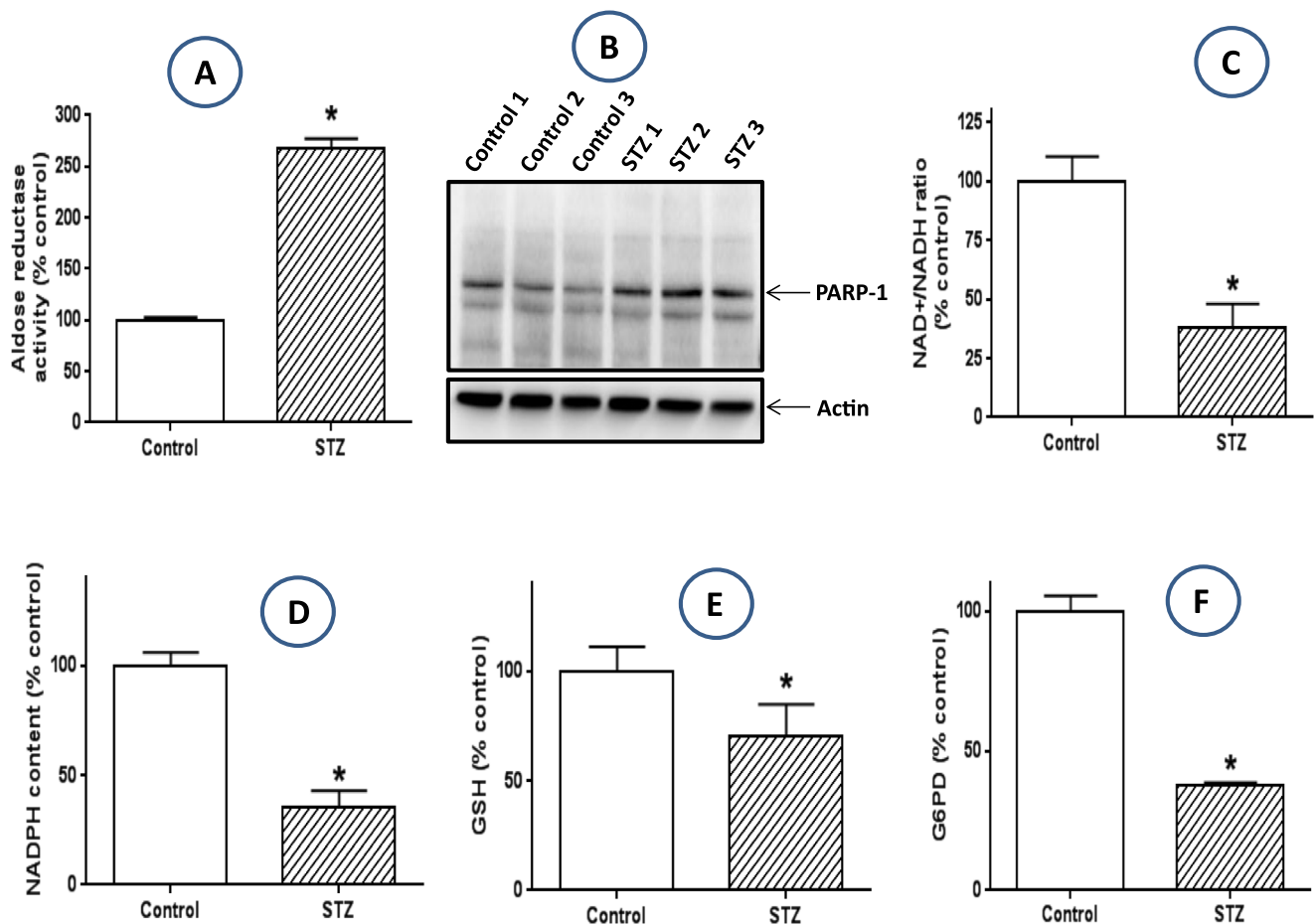


Fig. 1. : Redox imbalance parameters in the diabetic lung. When compared with the lungs from non-diabetic rats, the diabetic lungs show an increased aldose reductase activity (A), an increased protein expression of PARP-1, a significantly decreased level of NAD⁺(C), a decreased level of NADPH (D), a decreased level of reduced form of glutathione (GSH) (E), and a decreased activity of G6PD (F).

can be over-activated by hyperglycemia induced DNA oxidative damage, resulting in potential depletion of NAD⁺[48]. Therefore an overall outcome of NADH/NAD⁺ redox imbalance occurs in diabetic tissues [13]. To test whether this redox imbalance mechanism takes place in the diabetic lung, we measured aldose reductase activity and the protein content of PARP-1, results in Fig. 1A demonstrate that the activity of aldose reductase, the rate-limiting enzyme of the polyol pathway [49], was indeed higher than that in the control lung. Similarly, results in Fig. 1B demonstrate that PARP-1 protein content was upregulated in the diabetic lung. Together, the upregulation of these two pathways contributed to a much lower level of NAD⁺ in the diabetic lung than in the healthy lung as observed in Fig. 1C. Moreover, we also found that in the diabetic lung, NADPH content was lower, so was GSH content, the normal level of the latter depends on a normal level of NADPH that is used by glutathione reductase to make GSH from GSSG [50]. Additionally, we also found that the activity of glucose-6 phosphate dehydrogenase (G6PD) was also lower in the diabetic lungs than in the healthy lungs, suggesting that a decreased level of NADPH could be partly driven by a low activity of G6PD that is responsible for NADPH production from glucose [51,52].

3.2. Increased activities of mitochondrial electron transport chain complexes

The excess NADH in the diabetic lung as measured in Fig. 1C suggests that there is an oversupply of electrons to mitochondrial electron transport chain. Therefore we next determined the effects of this excess NADH on the enzyme activities of mitochondrial electron

transport chain components complexes I to IV. Results shown in Fig. 2(A-D) indicate that the enzyme activities of all the four complexes were elevated in the diabetic lung, suggesting an enhanced electron transport imposed by excess NADH on the mitochondrial electron transport chain. We also measured complex V activity but did not detect any different between control and diabetes (Fig. 2E). Nonetheless, ATP content was much lower in the diabetic lung than in the healthy lungs (Fig. 2F). These results suggested that the upregulated electron transport chain activities (I to IV) are not for ATP production. Rather, the increased electron transport chain function may contribute to increased ROS production, given that majority of ROS can be produced by mitochondria in diabetes [53].

3.3. Increased oxidative stress in the diabetic lung

To test the above hypothesis that the upregulated mitochondrial electron transport chain leads to increased ROS production, we then measured hydrogen peroxide in the lung homogenate. A comparison between control and diabetic lungs indicates that H₂O₂ content was much higher in the diabetic lung than in the healthy lung (Fig. 3A). Moreover, we also measured lung mitochondrial protein carbonylation using western blot assay. Results indicate increased total protein carbonylation in the diabetic group than in the control group (Fig. 3, C and D). Moreover, we also measured the activity and content of NQO1, a second phase antioxidant enzyme that is usually upregulated by the Nrf2 transcription factor signaling pathway [54]. Result in Fig. 3B indicates that both NQO1 content and activity were much lower in the diabetic lung than in the control lung. Taken together, our results

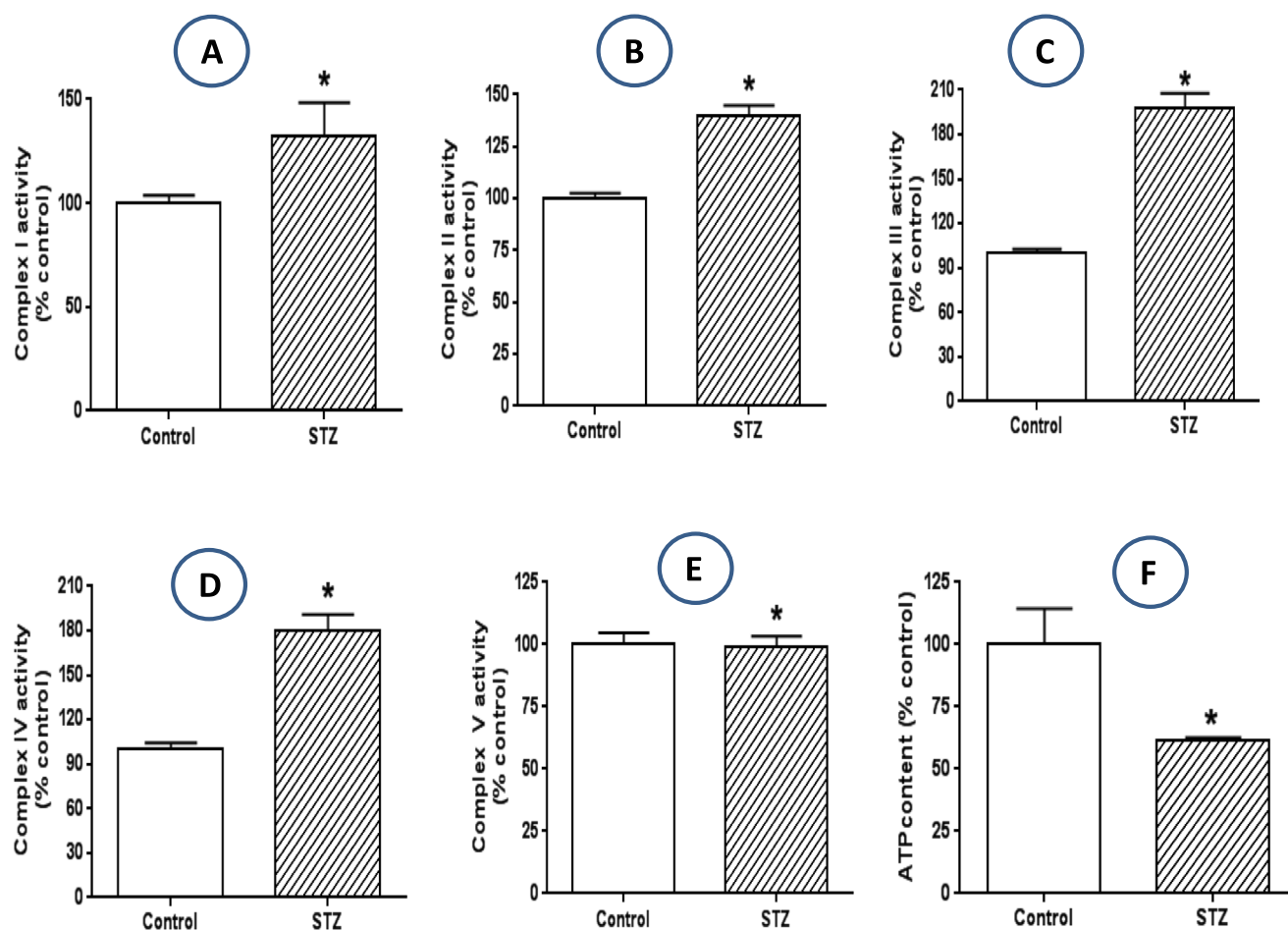


Fig. 2. : Activities of mitochondrial membrane complexes. The activities of mitochondrial electron transport chain complexes I to IV (A to D) all exhibited decreases in the diabetic lung when compared with those in the controls. No difference in complex V activity between control and STZ-diabetes could be detected (E). Cellular ATP content was lower in the diabetic lung than in the non-diabetic lung (F).

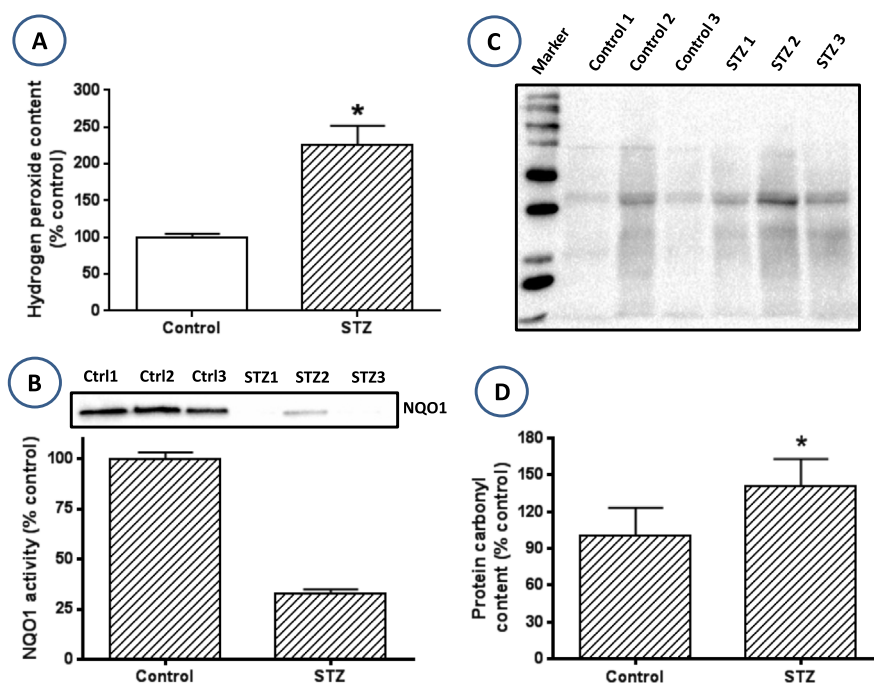


Fig. 3. : Elevated level of oxidative stress and attenuated antioxidative capacity in the diabetic lung. (A) ROS production reflected by the level of H₂O₂ was increased in the diabetic lung. (B) NQO1 protein content was lower in the diabetic lung than in the non-diabetic lung. (C) Lung mitochondrial protein carbonylation assessed by Western blot assay using aldehyde-reactive probe as the labeling reagent. (D) Densitometric analysis of protein carbonyl content between control and diabetes. Data are derived from (C).

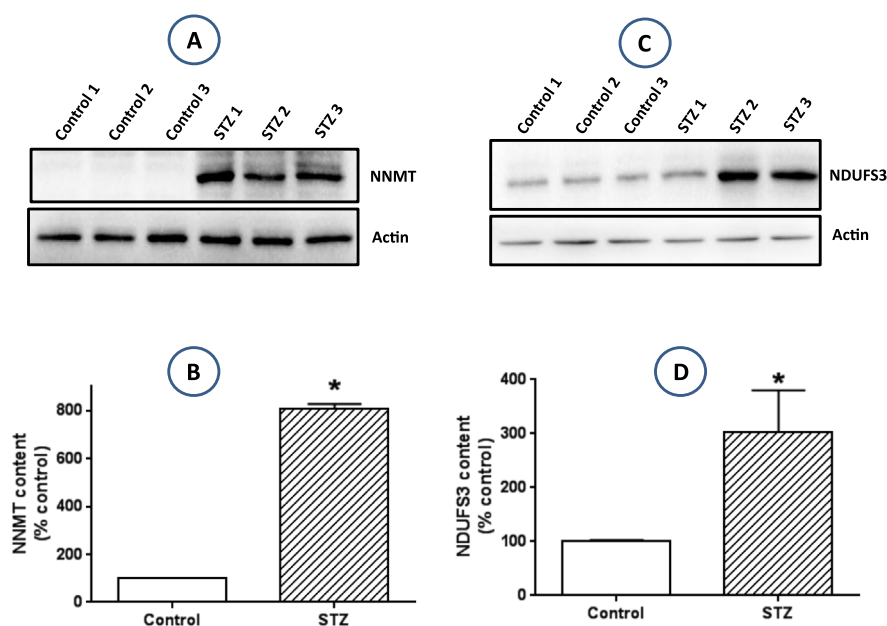


Fig. 4. : Evaluation of protein expression of NNMT and NDUFS3. (A) Increased expression of NNMT in the diabetic lung measured by Western blot assay. (B) Densitometric quantitation of Western blot band intensity shown in (A). (C) Increased expression of NDUFS3 in the diabetic lung measured by Western blot assay. (D) Densitometric quantitation of Western blot band intensity as shown in (C).

indicate that NADH/NAD⁺ redox imbalance in the diabetic lung induces oxidative stress that may be implicated in lung dysfunction in diabetes.

3.4. Upregulation of complex I activity is likely mediated by increased expression of nicotinamide N-methyltransferase (NNMT) and NDUFS3

As complex I is the entry point of electrons into the electron transport chain, we next determine whether hyperglycemia upregulates complex I. While complex I upregulation could be an adaptive response to excess NADH, this upregulation could also increase mitochondrial ROS production given that the more NADH oxidized by complex I, the more ROS produced by complex I [55,56]. Based on reports in the literature that NNMT can be upregulated by overnutrition and hyperglycemia and that this upregulation elevates the expression of the complex I subunit NDUFS3 [57–59] thereby increasing complex I activity, we measured by western blot methods the protein content of both NNMT and NDUFS3 (Fig. 4A and C). Densitometric analysis of these western blot results indicated that the protein content of both NNMT and NDUFS3 were significantly increased (Fig. 4B and D), demonstrating that NNMT upregulation by diabetic hyperglycemia can increase complex I activity by increasing the expression of complex I NDUFS3 subunit.

3.5. Attenuated expression of dihydrolipoamide dehydrogenase (DLDH) in the diabetic lung

Dihydrolipoamide dehydrogenase (DLDH) is a component of three mitochondrial alpha keto acid dehydrogenase complexes [37]. It is a key enzyme in the production of acetyl-CoA from pyruvate and branched chain amino acids [60]. DLDH uses NAD⁺ as one of its two substrates and makes NADH [61]. It is known that the level of NAD⁺ could affect either the level or the activity of DLDH [62,63]. To test whether a low NAD⁺ content in the diabetic lung leads to an attenuated DLDH function or a low DLDH protein content, we measured DLDH content and enzyme activity. Enzymatic activity was measured by both blue native gel electrophoresis and spectrometry. Results in Fig. 5A and B indicate that DLDH activity was indeed significantly lower in the

diabetic lung than in the healthy lung. Moreover, data in Fig. 5C indicates that DLDH content was remarkably decreased in the diabetic lung, indicating that a lower DLDH activity in the diabetic lung is contributed by a lower DLDH content. Furthermore, when the DLDH activity bands on the blue native gel were analyzed by mass spectrometry, 7 DLDH peptides were recovered in the control samples while none could be recovered in the STZ-treated samples (data not shown), confirming that DLDH is indeed down-regulated in the diabetic lung.

It should be pointed out that the low DLDH activity observed in A and B could also be contributed by DLDH acetylation as DLDH from the diabetic lung was found to exhibit elevated levels of protein acetylation (Fig. 5D, upper panel). Interestingly, DLDH protein was not found to be damaged via carbonylation (Fig. 5D, lower panel), a parameter used to quantitate protein oxidative damage [44,64]. This finding is in agreement with our previous findings that DLDH is not an apparent target for carbonylation during aging [61], a process associated with increased oxidative stress [65].

3.6. Decreased mitochondrial sirtuin 3 (sirt3) content in the diabetic lung

It has been well established that the level of NAD⁺ in a cell can dictate the level of sirtuin proteins [66], which are deacetylation enzymes that are known to be involved in redox signaling and metabolic control [67]. Our finding that NAD⁺ content is decreased in the diabetic lung suggests that sirt3 level could be attenuated as well given that sirtuin protein expression is NAD⁺ dependent [66]. To test this likelihood, we measured mitochondrial sirt3 protein content by western blot. Result in Fig. 6A demonstrates that sirt3 protein content was severely decreased in the diabetic lung than in the control lung. Consequently, total mitochondrial protein acetylation in the diabetic lung was greater than that in the control lung (Fig. 6B and C), which is in agreement with the observation that DLDH acetylation was increased in the diabetic lung (Fig. 5D, upper panel).

3.7. Impaired mitochondrial membrane potential and increased cell death in the diabetic lung

Our findings that enhanced mitochondrial electron transport did

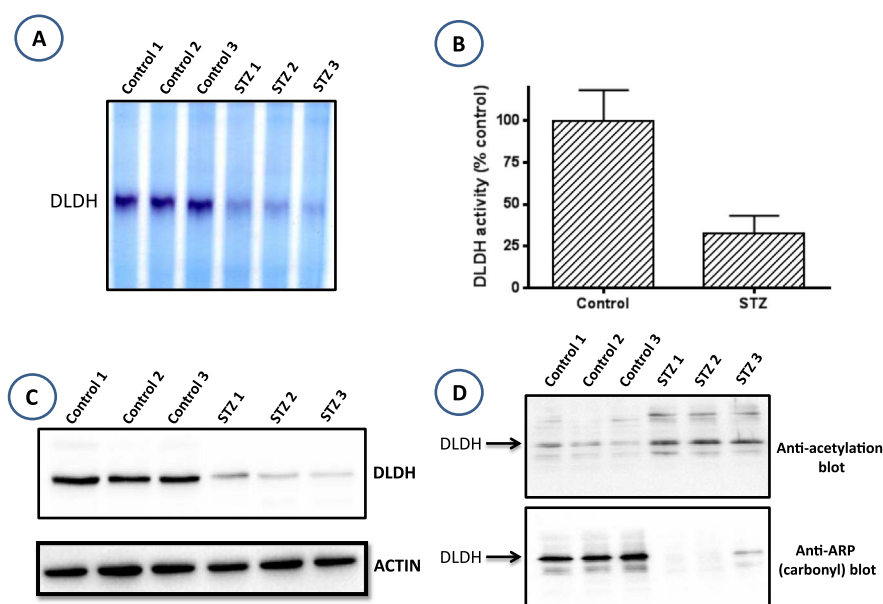


Fig. 5. Comparison of DLDH activity and expression between control and diabetic lungs. (A) Decreased DLDH activity in the diabetic lung assessed by BN-PAGE. (B) Decreased DLDH activity in the diabetic lung measured spectrophotometrically. (C) Decreased DLDH protein content in the diabetic lung assessed by Western blot assay. (D) DLDH acetylation (Upper), but not DLDH protein carbonylation (lower) was increased in the diabetic lung. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article)

not lead to increased mitochondrial ATP production suggest that mitochondria in the diabetic lung are not well-coupled. Therefore, we next measured mitochondrial membrane potential. Results in Fig. 7A demonstrate that diabetic pulmonary mitochondrial membrane potential was lower than that in control, which could lead to enhanced electron leakage and oxidative stress as shown in Fig. 5. Moreover, less mitochondrial ATP production can also suggest an increased cell death. Indeed, measurement of caspase-3 activity, a parameter reflecting the magnitude of cell death, demonstrates an increased level of apoptosis as caspase-3 activity was much higher in diabetes than in control (Fig. 7B).

4. Discussion

The major findings of the present study are the following: 1) NADH/NAD⁺ redox imbalance occurred in the diabetic lung and was likely contributed by activation of the polyol pathway and PARP. 2) The activities of mitochondrial complexes I to IV were upregulated while there were no changes in complex V activity. 3) Mitochondrial ATP output was lower in the diabetic lung than in the non-diabetic lung. 4) Mitochondrial ROS production and protein carbonylation were increased, which was accompanied by decreased protein content and activity of NQO1, an inducible antioxidant enzyme involved in cellular defense against oxidative stress [68]. 5) Complex I upregulation by diabetic hyperglycemia was likely achieved by upregulation of NNMT

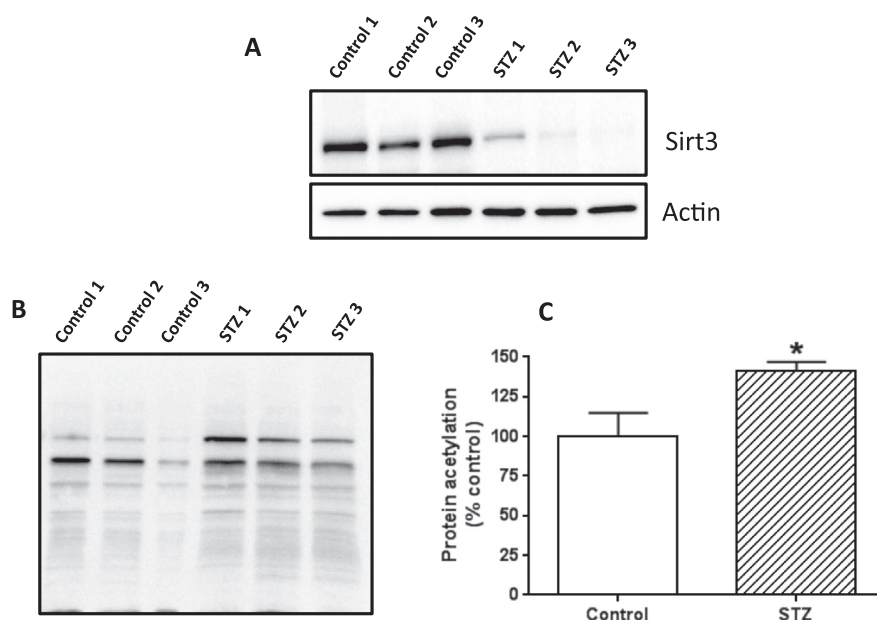


Fig. 6. (A) Sirt3 protein content was decreased in the diabetic lung. Anti-sirt3 antibodies were used for this evaluation with actin as the loading control. (B) Western blot detection of mitochondrial protein acetylation; shown are lung mitochondria isolated from three control rats and 3 diabetic rats, respectively. (C) Densitometric quantification of mitochondrial protein acetylation. Data were derived from (B).

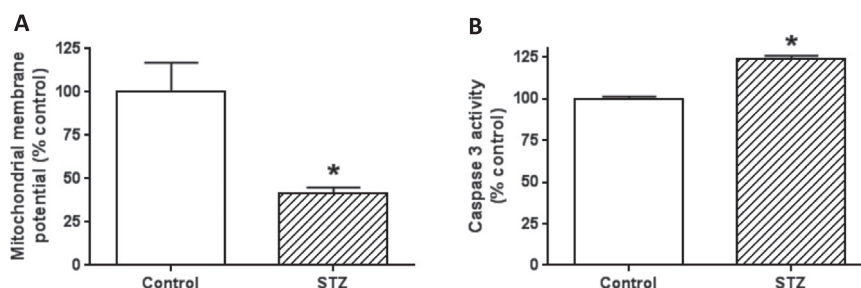


Fig. 7. : Determination of mitochondrial membrane potential and cell death. (A) Mitochondrial membrane potential in the diabetic lung was significantly lower than that in the healthy lung. (B) Increased caspase-3 activity in the diabetic lung than in the healthy lung, indicating increased cell death in the diabetic lung (N = 3 for each measurement).

that in turn upregulates NDUFS3, a key complex I subunit involved in complex I assembly and function [69]. 6) DLDH protein content was decreased in the diabetic lung, which impaired DLDH activity that may also be accentuated by the observation that DLDH acetylation was increased in the diabetic lung, a process that can be enhanced by down regulation of mitochondrial sirt3 that is responsible for protein deacetylation [70]. 7) Mitochondrial membrane potential in the diabetic lung was decreased and cell death was increased. Taken together, results of the present study shed insights into the biochemical mechanisms of lung injury in diabetes. It should be pointed out that one caveat of the study is that we did not study whether the redox imbalance occurs to all the cellular populations in the lung that is composed of nearly 40 different cell types.

Our study presents strong evidence that there occurs also redox imbalance in the diabetic lung with NADH being in excess. Excess NADH could over burden the electron transport chain and cause more mitochondrial ROS production. As an adaptive response to handle NADH pressure complex I was found to be upregulated (Fig. 2A), together with other electron transport chain complexes II to IV (Fig. 2B–D). The adverse effect of this upregulation, unfortunately, would be increased ROS production given that the more NADH oxidized by complex I, the more ROS produced by complex I [26,71]. Indeed, excess NADH appears to be used for ROS generation as complex V was not upregulated and ATP output by mitochondria was decreased (Fig. 2E and F), indicating an uncoupling effect of diabetic hyperglycemia on pulmonary mitochondrial oxidative phosphorylation as shown in Fig. 7A and increased cell death as shown in Fig. 7B. These results demonstrate overall mitochondrial abnormalities in the presence of glucose oversupply in that excess NADH is not completely used for ATP production but rather diverted for production of mitochondrial ROS that could be involved in cell death and lung injury in diabetes.

Our study also demonstrates that NADPH-related signaling pathways in the diabetic lungs were also compromised. Not only NADPH level was found to be attenuated, activity of G6PD and levels of GSH (reduced glutathione) were also found to be suppressed. It seems that the decrease in NADPH content could be contributed by two pathways that are deregulated in diabetes by hyperglycemia. One pathway is NADPH consumption by the polyol pathway for the generation of NADH; another pathway is functional impairment of G6PD that could lead to less NADPH production. As NADPH is required for GSH formation from GSSG by glutathione reductase [42,72], less NADPH would lead to less GSH formation. This is indeed what we have found in the diabetic lung whereby GSH content was low (Fig. 1E). Our results are similar to what have been found in the diabetic kidneys in which G6PD activity, NADPH and GSH levels were all found to be lower in diabetic animals than in non-diabetic controls [73]. It should be noted that the alterations in G6PD activities in diabetes are likely tissue dependent. For example, in the brain of STZ diabetic rats, G6PD activity was markedly increased [74]. G6PD activity was also found to be increased in the liver of Zucker diabetic rats [75]. Similarly, NADPH content in certain diabetic tissues has been reported to be elevated

when compared with that in non-diabetic conditions [76–78]. Nonetheless, in our present study, we found that NADPH level declined in diabetic lung (Fig. 1D). It should be pointed out that the level of NADH can increase dramatically in the presence of a decreased NADPH content is due to the fact that cellular NADPH concentration is usually much higher than that of NADH. For example, in red blood cells, NADPH content is approximately 10 times higher than that of NADH [79].

In the present study, we also analyzed NAD⁺-dependent enzymes such as NQO1, DLDH, and sirt3. Our results show that the functions of all the three enzymes were impaired in the diabetic lung, either through down regulation of protein expression or posttranslational modifications or both. With respect to NQO1, as its expression is controlled by Nrf2 transcription factor [80], our observation that NQO1 content showed a decrease in the diabetic lung suggests that the Nrf2 signaling pathway is suppressed in the diabetic lung; which needs to be further evaluated in future studies. Nonetheless it has been reported that the Nrf2 signaling pathway is down regulated in other diabetic tissues such as liver and heart [81, 82]. It should be noted, however, that how Nrf2 is regulated in diabetes may be tissue dependent as it has been shown that in the kidney, Nrf2 could be upregulated by diabetic hyperglycemia [83].

With respect to DLDH, this protein is also known to be able to generate ROS [84–86]. This enzyme uses NAD⁺ as its substrate and makes NADH that can be fed into the electron transport chain. In the presence of excess NADH, DLDH can be inhibited via a feedback inhibitory mechanism [87]. In the present study, we found that DLDH in the diabetic lungs was down regulated with a diminished DLDH protein content (Fig. 5C). This diminution would certainly impair the role of DLDH in mitochondrial bioenergetics. Functional impairment of DLDH in the diabetic lung could also partially originate from its cysteine acetylation; a process regulated by sirt3 and is known to affect protein functions [70,88,89]. Our findings that DLDH underwent protein acetylation in the diabetic lung are in agreement with previous reports that DLDH is a target of protein acetylation [88,90,91]. We think that DLDH acetylation in diabetic lung could be governed by two mechanisms. One is due to oversupply of acetyl-CoA that can chemically modify a protein cysteine residues [92–94], another mechanism would be the down regulation of mitochondrial sirt3 (Fig. 6) detected in the diabetic lung, which would lead to less deacetylation of DLDH. Our data suggest that DLDH dysfunction might be a pathogenic mechanism for diabetic lung injury.

With respect to sirt3, our observation that sirt3 is down regulated in the diabetic lung mitochondria (Fig. 6) agrees with the results of previous studies whereby sirt3 shows a decreased expression in diabetic tissues [95–98]. The reason for this is likely due to the fact that NAD⁺ is decreased in diabetes [99,100]. It is known that the level of sirtuin expression is dependent on NAD⁺ content [66,101]. As sirt3 is responsible for protein deacetylation, its decreased expression would certainly increase protein acetylation, as was in the case for DLDH and other mitochondrial proteins (Fig. 6B and C). Therefore, impaired sirt3 signaling pathway could provide another mechanism of mitochondrial

abnormalities associated with lung injury in diabetes.

5. Summary

In this study, we have presented evidence that in the diabetic lung, NADH/NAD⁺ redox balance was perturbed with NADH being in excess and NAD⁺ being deficient. Consequently, mitochondrial electron transport chain could be under NADH electron pressure and was indeed found to be upregulated in response to this pressure. However, this upregulation does not seem to lead to enhanced mitochondrial ATP production as complex V activity was not upregulated and ATP content was suppressed. Conversely, the upregulation of electron transport chain function was found to be associated with decreased mitochondrial membrane potential, increased ROS generation, elevated oxidative stress, and increased cell death. Moreover, the function of NAD⁺-dependent enzymes such as NQO1, DLDH, and sirt3 were all found to be impaired in the diabetic lung. Taken together, the present study has elucidated mechanisms by which lung function can be impaired in diabetes.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

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